

Immune Deficiency As a Risk Factor in Epstein-Barr Virus-Induced Malignant Diseases

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Epstein-Barr virus (EBV) is a ubiquitous DNA virus that normally infects silently, establishing lifelong latency. Substantial empirical observations support the view that immunodeficiency is permissive in EBV-induced lymphoproliferative diseases (LPD). Primary immune deficient patients such as those with X-linked lymphoproliferative disease and individuals with acquired immune deficiency secondary to immunosuppressive drugs for organ transplantation or individuals infected with human immunodeficiency virus are also at very high risk for lethal LPD. The importance of immunodeficiency and EBV in the development of head and neck carcinomas and uterine cervical carcinoma is less clear. Methods are available for detecting immunodeficiency and EBV genome and thus preventive strategies are being developed to preclude LPD from occurring.

Introduction

Milestones in the discovery of Epstein-Barr virus (EBV)-induced diseases include description of Burkitt's lymphoma (BL) by Denis Burkitt in 1958, the discovery of the EBV 25 years ago by Anthony Epstein, Burt Achong, and Yvonne Barr, the serological association of EBV with nasopharyngeal carcinoma (NPC) by Lloyd Old in 1966, and the discovery that EBV causes infectious mononucleosis (IM) by the Henles in 1968 (1).

Until about the mid-1970s, the two prevailing hypotheses regarding the oncogenicity of EBV included the notion that benign and malignant strains of EBV prevailed or, alternatively, that immunodeficiency played a major role in the development of certain malignancies (2). Supporting the latter hypothesis was our discovery in 1975 of the X-linked lymphoproliferative disease (XLP) (3), wherein affected males develop fatal IM, malignant lymphoma, or acquired agammaglobulinemia following infection by EBV. Furthermore, XLP provided clues to the cause of malignant lymphoma found earlier by Starzl in renal transplant recipients (4), and Gatti and Good also had found a very high prevalence of malignant lymphoma in immune-deficient children (5).

Summarized herein are the roles of immunodeficiency in the development of EBV-induced lymphoproliferative diseases (LPD). The mechanisms responsible for the evolution of these diseases at cytogenetic and molecular levels and the findings of EBV-carrying carcinomas involving the head and neck and uterine cervix are described. A brief background including the molecular anatomy of EBV and normal immune responses to the virus is outlined.

Immunobiology of EBV

EBV is a DNA virus of 172 kb. It gains access to the human epithelium in the oropharyngeal region through CR2 receptors. Within the squamous epithelium, the virus is able to undergo productive infection. Intimate association of the lymphoid tissues with the oral epithelium permits infection of the human B-cell, which can be immortalized (6). Most individuals become asymptotically infected during childhood, and lifelong latency is established.

The virus is composed of about 100 genes, which are currently being mapped (Fig. 1). Six different types of EBV-determined nuclear antigen (EBNA) and two types of early antigens (EA) have been identified. EBNA-1 maintains expression of EBV in the immortalized cell in the extrachromosomal plasmids. The immortalizing or transforming capacity of EBV resides in EBNA-2 and possibly in latent membrane protein (LMP) (7). The LMP acts as an oncogene in two established rodent lines. Both EBNA-2 and LMP dramati-

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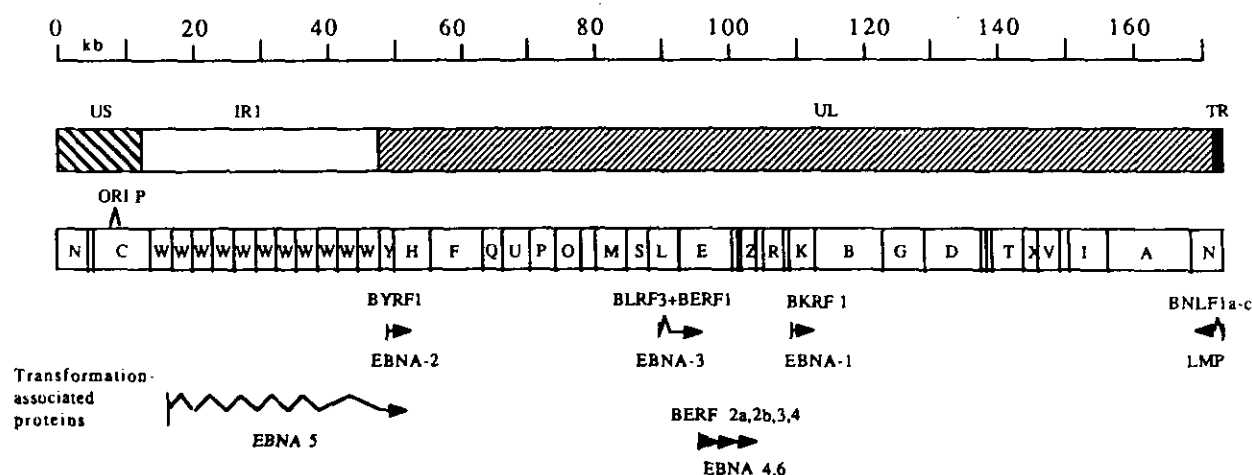


FIGURE 1. The EBV DNA and the coding sequences of the transformation-associated proteins. (Top) relative size in kilobase pairs. (Upper bar) organization of the genome in short unique sequence (US), internal repeat (IR), long unique sequence (UL), and terminal repeats (TR). (Lower bar) positions of the Bam HI restriction fragments. (Gene map) size, position, and direction of transcription are given by arrows. The names of the open reading frames are given above each transcript. For example, BYRF1 stands for Bam Y rightward open reading frame number 1. EBNA, EBV-determined nuclear antigen; LMP, latent membrane protein. From Torsteinsdottir (46).

cally activate expression of adhesion molecules LFA-1, LFA-3, and ICAM-1 and CD23 FCR11 and transferrin receptor on lymphoblastoid cells (?).

Silently, EBV infects children establishing lifelong latency; however, when infected primarily, two-thirds of adolescents develop IM. The clinical and pathological features of IM are the result of the immunological struggle going on between the EBV-infected B-cells and T-cells, which are both polyclonally stimulated by the infection. Hence, pharyngitis, fever, enlarged lymph nodes, and hepatosplenomegaly are frequent signs and symptoms of IM (6).

The atypical lymphocytosis seen during the initial few weeks of IM are due to a polyclonal expansion of predominantly CD8-positive T-cells. Also, a modest increase in both CD4 and SIg-positive B-cells is noted initially during the illness (8). Both EBV-specific and nonspecific cytotoxic T-cells and natural killer cell activity contribute to the elimination of EBV-infected B-cells. During latency, approximately one in one million B-cells in peripheral blood contain EBV. Thus, spontaneous cell lines can be established from peripheral blood *in vitro*.

EBV-specific antibodies sequentially emerge following infection including IgM anti-VCA, IgG anti-VCA, anti-EA, and after approximately 2 months, IgG anti-EBNA. Congenitally immune-deficient children tend to show low or absent anti-EBNA response, and depending on the type of immune deficiency, anti-EA and anti-VCA titers may be abnormally elevated (9). Presence of EBV can be identified using a variety of techniques (Table 1).

EBV-Induced LPD in Immune Deficient Patients

From 1979 to 1981, Purtilo initiated collaborative studies with groups of investigators in Stockholm, Min-

Table 1. Detection of EBV in patient samples.

Polymerase chain reaction using synthetic oligonucleotide EBNA primers
Southern blot hybridization using cloned fragments of EBV DNA
<i>In situ</i> hybridization using cloned fragments of EBV DNA
Development of EBV-positive spontaneous lymphoblastoid cell lines
EBNA staining of infected cells
Immunoblotting for presence of EBNA proteins
EBV-specific serology using IF or ELISA techniques
Rapid slide tests
Heterophile antibodies
Immortalization of cord lymphocytes with throat washings

neapolis, and elsewhere to determine whether EBV is responsible for LPD in organ transplant recipients and children with primary immunodeficiency. The results of these collaborative studies were published in a special edition of *Cancer Research* in 1981 (10) and in a monograph (11).

These collaborative studies disclosed the LPD found in renal transplant recipients is due to EBV. In young patients, LPD tends to be disseminated but with time can evolve into a malignant lymphoma (12). This is likely due to primary infection occurring in the young patients leading to an IM-like disease. In contrast, elderly individuals who have latent virus undergo reactivation of EBV owing to immunosuppression. These patients are more apt to develop solitary masses in extranodal sites.

Recognition that EBV is the cause of LPD in organ transplant recipients has permitted development of strategies to prevent and treat individuals with this complication. For example, withdrawal of immunosuppression can lead to regression of the LPD in cases where the proliferation is polyclonal (13). Furthermore, use of acyclovir can be beneficial in some instances (12). Early diagnosis of LPD can also lead to prevention of the malignant lymphoma (Table 1).

The use of high-dose cyclosporin and monoclonal an-

tibody to CD3 renders individuals at very high risk for malignant lymphoma. Particularly vulnerable are bone marrow transplant recipients who are treated with OKT3 (corresponding to CD3) monoclonal antibody to deplete donor marrow T-cells (14). Methods must be developed to obviate these deadly consequences of immunosuppression.

Individuals infected with human immunodeficiency virus (HIV) are at high risk for developing EBV-induced diseases including hairy leukoplakia of the tongue, persistent lymphadenopathy, pulmonary lymphoid interstitial pneumonia (LIP), hyperplasia of the lymphoid tissue in the colon, and malignant B-cell lymphomas. An estimated 1,000-fold increase of B-cell lymphomas occurs in individuals infected with HIV (15). Approximately one-half of these lymphomas carry EBV genome. Similarly, the LIP lesion in the lungs of children with AIDS is due to EBV. It is likely that long-term survivors with HIV infection will be at increasingly high risk of developing EBV-driven LPD.

We and others have demonstrated that children with immunodeficiency diseases have a markedly increased risk for EBV-driven LPD. This ranges from about 2% for children with Bruton's agammaglobulinemia to about 25% for males with XLP (16). Our laboratory focuses on XLP, and so a summary of the disease will be provided as a model for studying immunodeficiency and EBV-induced LPD.

LPD in Patients with XLP

During the two decades that have passed since Purtilo performed an autopsy on an 8-year-old boy in the Duncan family who had succumbed to IM, much has been learned about XLP. Our international registry of XLP now contains 60 kindreds who are being studied comprehensively and prospectively.

Approximately 240 individuals are enrolled in the registry. Patients with XLP invariably develop severe or fatal IM (60% of cases), acquired hypogammaglobulinemia (30% of cases), and/or malignant lymphoma (25% of cases). Phenotypes may overlap in the same individual or occur sequentially with time. Approximately 60% of the boys succumb to IM by 10 years of age, and all individuals with the XLP genotype die before they are 40 years old (17).

The fatal infectious mononucleosis (FIM) phenotype is characterized by fulminating uncontrolled polyclonal T- and B-cell proliferative responses following EBV infection. Numerous EBV-infected B-cells infiltrate liver, bone marrow, and other organs. Misdirected cytotoxic T-cells concurrently invade the tissues but fail to eliminate the EBV-infected B-cells and thus miss their target, possibly causing damage to the liver, bone marrow, and other infiltrated tissues. Consequently, the individuals succumb to liver (18) and/or bone marrow failure (19). Individuals who survive primary EBV infection and acute infectious mononucleosis subsequently develop either hypogammaglobulinemia or malignant B-cell lymphoma. Those with the hypogammaglobulinemia

phenotype often show marked necrosis of lymph nodes, thymus, and other lymphoid tissues. Although their peripheral blood B-cells are present in normal numbers, they fail to secrete adequate amounts of immunoglobulin.

The males who develop malignant lymphoma following EBV infection generally have the BL-like type involving the ileocecal region, central nervous system, or other extranodal sites (20). Survival is often long-term, especially in those who concurrently exhibit hypogammaglobulinemia (17).

Recently we have mapped the XLP locus using DNA probes in the X chromosome, especially DXS42, DXS37, and DXS10. These probes have restriction length polymorphisms (RFLP) and link tightly with the XLP locus (21). We have also identified a family in Omaha with a deletion involving the XLP locus at Xq25 (22). These findings allow us to make accurate diagnoses and to provide genetic counseling and immunoprophylaxis with IV immunoglobulin containing antibodies to EBV.

Mechanisms of Polyclonal to Monoclonal B-Cell Conversion

Klein (23) has hypothesized that EBV drives polyclonal B-cell proliferation and that breakage and reciprocal translocation involving chromosomes 8 (*c-myc*), 14 (heavy chain), 22 (lambda), and 2 (kappa) are involved in development of BL. Klein and colleagues have also demonstrated a similar condition in rats and mice plasmacytomas. Moreover, a molecular construct of *Ig-myc* when in transgenic mice results in malignant pre-B and B-cell lymphomas in the mice. These mice are patented and can be purchased for studies.

That immunodeficiency is permissive of EBV-induced malignant lymphoma is attested to by studies demonstrating immunodeficiency and EBV-containing malignant lymphoma in children infected with malaria and EBV in the BL belt, organ transplant recipients, AIDS victims, and immune-deficient children (24). Furthermore, recent studies in the severe combined immunodeficiency (SCID) mouse demonstrate that when peripheral blood mononuclear cells are engrafted from an EBV seropositive individual, the mice eventually develop lethal LPD (25). The foregoing conditions are all characterized by T-cell deficiency allowing EBV to drive B-cell proliferation in a sustained manner.

EBV-Induced Carcinomas

The EBV genome was first found in 1970 by Zur Hausen (26) in nasopharyngeal carcinoma (NPC) and subsequently Brichacek has demonstrated the virus in supraglottic tumors and tonsillar carcinomas (27,28) and occasional thymomas (29). Our inability to explain how EBV infection during infancy could be responsible for NPC in adults has been perplexing. Surely, immunologic and genetic factors play a role in allowing NPC to develop. The tumor develops primarily in Chinese males. Males have a relative immune deficiency com-

Table 2. Expression of EBNA and LMP in Burkitt's lymphoma (BL) tissues, lines, and lymphoblastoid cell lines.

EBNA and LMP	BL tissue	BL line	Lymphoblastoid cell lines
EBNA			
1	+	+	+
2	-	-/+	+
3	-	-/+	+
4	-	-/+	+
5	-	-/+	+
6	-	-/+	+
LMP	-	-/+	+

pared to females (30). Cofactors such as nitrosamines and phorbol esters have been proposed as activators of EBV leading to the NPC. Supporting the view that EBV is responsible for the tumor is the finding of monoclonality of the EBV incorporated in the tumors (31).

Recently, investigators in Birmingham, Memphis, and Omaha have focused attention on EBV as a potential factor in uterine cervical carcinogenesis. Young and Sixbey (32) have infected cervical epithelial cells, and the virus has been isolated from mucus from the endocervix. More recently, Luka and others have found EBNA staining of cervical intraepithelial neoplasia and precursor lesions (J. Luka, personal communication). Moreover, we have found defects in natural killer cell activity and abnormally elevated EBV titers in patients with cervical carcinoma (D. Purtilo et al., unpublished observations). Few would argue that human papillo-

mavirus (HPV) is important in cervical carcinogenesis (33). EBV, however, may be a cofactor in the induction of this malignant neoplasm. This hypothesis is being tested actively in our laboratories.

Cellular Regulation of Expression of EBV-Encoded Proteins

Evidence is substantial that tumor cells differentially express EBV-encoded proteins. EBNA1 is expressed in all virus genome-carrying cells and is probably responsible for maintenance of the virus genome in the episomal state (7). EBNA2 is connected with the transforming capability of the virus, since variant strains such as P3HR1 that lack this part of the genome do not immortalize B-cells *in vitro*. The BL lymphoblastoid cells derived from the same donor are differentially resistant to cytotoxic T-cells. BL cells are probably resistant because they do not express EBNA2-6 and LMP. At least EBNA2 and LMP may serve as targets for cytotoxic T-cells after they have been processed to antigen peptides and associated with MHC class I (34). Moreover, BL cells downregulate certain class I HLA determinants as compared to the lymphoblastoid cell lines (35-37). Finally, BL cells express certain cell adhesion molecules at lower levels than the corresponding lymphoblastoid cells (38). Klein has speculated that these differences between the BL and the lymphoblastoid cells provide immunoselection advantage with resistance to cytotoxic T-cells (Table 2).

Table 3. Spectrum of classical EBV-associated diseases and disorders in immunologically compromised patients.

Classical malignancies	Complications of IM (acute, recurrent, or chronic)
BL	Hepatitis
NPC (undifferentiated type)	Rupture of spleen
Unusual malignancies	Virus-associated hemophagocytic syndrome
Tonsil carcinoma	Hemolytic anemia
Supraglottic laryngeal carcinoma	Aplastic anemia
Thymic carcinoma	Agranulocytosis
Salivary gland carcinoma	Erythroblastopenia
Chronic myeloproliferative disorder?	Thrombocytopenia
Disorders in primary immunodeficiencies	Agammaglobulinemia or hypogammaglobulinemia
Malignant B-cell lymphoma or lymphoproliferation (primarily)	Neurological disorder (meningoencephalitis, transverse myelitis, Guillain-Barre syndrome, Bell's palsy, cerebellar ataxia, and syndrome of inappropriate antidiuretic hormone secretion)
XLP ^a	Arthritis
Ataxia telangiectasia	Myocarditis
Wiskott-Aldrich syndrome	Pneumonitis
Severe combined immunodeficiency	Nephritis
Common variable immunodeficiency	Parotiditis
Chediak-Higashi syndrome	Complications of chronic active EBV infection
Selective IgM deficiency	Lymphadenopathy
Disorders in transplant recipients (renal, cardiac, lung, liver, bone marrow, and thymic epithelium)	Hepatosplenomegaly
Malignant B-cell lymphoma or lymphoproliferation	Pancytopenia
IM	Hypogammaglobulinemia
Allograft rejection	B- or T-cell malignant lymphoma or lymphoproliferation
Disorders in AIDS	Cervical carcinoma
Hairy leukoplakia	Miscellaneous
Lymphadenopathy	Reactivation in pregnancy
Malignant B-cell lymphoma	Birth defects?
Lymphoid interstitial pneumonitis	
Colonic lymphoid hyperplasia	

^a Patients with XLP have mainly exhibited severe or fatal IM, acquired hypogammaglobulinemia, and malignant B-cell lymphoma.

Prevention and Therapy Against EBV-Induced LPD

Based on the observation that children are protected from BL up until 6 months of age by EBV-neutralizing antibodies from their mothers, we have placed seronegative patients with XLP on IV immunoglobulin. In one instance, relatively high titer antibodies failed to protect against EBV infection with FIM ensuing (D. Purtilo, unpublished observations). We have also attempted to use interferon gamma (39), and more recently, high-dose immunoglobulin and interferon alpha, with limited success (40). Shapiro et al. (41) have successfully treated immunodeficient children with these agents.

Development of a vaccine against EBV membrane antigen (GP340) has resulted in some protection of cotton-topped tamarins against primary EBV infection (42-45). Logistical considerations seem to preclude extensive use of a vaccine in southeast Asia and tropical Africa; however, in congenitally immune-deficient patients such as those with XLP, vaccination may be of some benefit. Currently, Epstein is testing a vaccine in normal healthy volunteers (Anthony Epstein, personal communication).

Summary

In summary, the ubiquitous EBV usually does not cause disease in humans. However, in immune incompetent individuals, life-threatening lymphoproliferative diseases can arise. Early diagnosis and recognition of immune deficiency and EBV-driven LPD can be life saving. The various EBV-related diseases that we and others have identified are listed in Table 3. Clearly, immunodeficiency is a major risk factor in EBV-induced carcinogenesis. Immunodeficiency to other DNA viruses such as papilloma virus and hepatitis B virus may potentially permit squamous cell carcinoma and hepatocellular carcinoma to ensue in certain patients.

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